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AFLATOXIN PROBLEM IN CORN AND POSSIBLE SOLUTIONS

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INTRODUCTION

Of the wide array of identified, toxic, fungal metabolites or mycotoxins, the aflatoxins have been studied the most intensively. Expanding research effort developed especially after aflatoxins were recognized as substances that occur naturally in some commodities traditionally used for human food and animal feed. These toxins are highly carcinogenic in various animal species and at sublethal levels can dramatically reduce feed efficiency and the general health of domestic animals.

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Historically, aflatoxins were discovered in the early 1960's after an outbreak of a disease in turkeys was attributed to a fungal-contaminated peanut meal (14). The predominant fungus in the meal was *Aspergillus flavus* Link ex Fr. This organism is widely distributed in nature, occurring in soil and various plant residues. The genus *Aspergillus* is divided into a number of sections of closely related species; *A. flavus* is taxonomically associated with the larger *Aspergillus flavus-oryzae* group (43). Members of this group are broadly characterized by production of greenish-yellow spores and by the absence of ascospores.

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After the fungus in the contaminated peanut meal had been identified, four distinct fluorescing substances were isolated that appeared to be responsible for the disease in turkeys. The compounds were given the broad name of aflatoxins identifying their generic origin, and delineation of the four substances was made on the basis of their fluorescent color (B for blue and G for green) with subscripts relating the relative chromatographic mobility (Fig. 1). Aflatoxin M_1 is the hydroxylated derivative of B_1 produced by fungi and in mammalian metabolism of ingested toxin. The substance is rapidly excreted by animals in both urine and milk. The hazard arises from occurrence in milk since M_1 is approximately as toxic as B_1 (14, 36).

Since the initial observations of toxicity associated with *A. flavus*-contaminated commodities, aflatoxin production has been clearly established by a wide range of *flavus* isolates and strains of a closely related species, *Aspergillus parasiticus* Speare. Generally, the fungus in

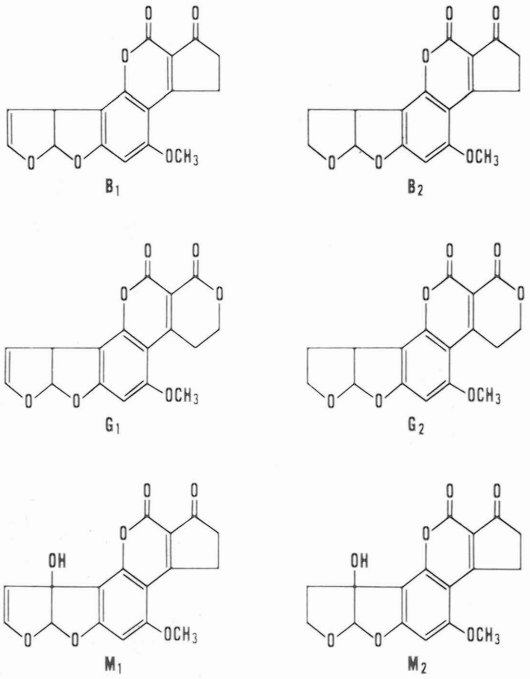

Figure 1. Structural formulas of various aflatoxins and aflatoxin derivatives.

foods and feeds naturally contaminated with aflatoxin is *A. flavus*. Its presence in a commodity is not always related to aflatoxin contamination since certain conditions are required for the organism to produce toxin and since some strains of *A. flavus* isolated from moldy substrates are unable to produce the toxin (14, 17).

AFLATOXIN TOXICITY AND CARCINOGENICITY

Acute toxicity studies have shown that many animal species are susceptible to aflatoxins with LD_{50} values ranging from 0.3 to 18 mg of toxins/kg body weight (Table 1) (14, 64). Factors affecting toxicity

TABLE 1—Single LD_{50} dose levels for Aflatoxin B_1

Species	LD_{50} (mg/kg)	Species	LD_{50} (mg/kg)
Rabbit	0.3–0.5	Monkey	2.2
Duckling	0.3–0.6	Sheep	2.0
Cat	0.6	Chick	6.5–16.5
Pig	0.6	Mouse	9.0
Rainbow trout	0.8	Hamster	10.2
Dog	1.0	Rat	5.5–18.0
Guinea pig	1.4–2.0		

Source: References 14, 64, 65.

include age, sex and nutrition of animals. Invariably, young animals are more susceptible than older ones, and males more susceptible than females. The typical toxicity syndrome associated with acute levels of aflatoxin includes liver damage characterized histologically as peripheral zone necrosis and bile duct proliferation (14, 64). These cellular changes in the liver are routinely identified as a precancerous state, but clear association between development of liver tumor and aflatoxin ingestion has only been shown in rats, mice, ducks, rainbow trout, ferrets and monkeys (2, 14, 65).

In addition to the well-documented cases of liver tumor, hepatoma, in some test animals, aflatoxin has also been implicated in neoplasms of the glandular stomach, lung, salivary gland, colon and skin (14, 65).

In observing dose-response relationship between aflatoxin and tumor development, Carnaghan (10) found that administration of a single LD_{50} dose of the toxin induced liver tumors in rats surviving held

without further treatment. Wogan and his colleagues (65, 66) fed rats rations containing various levels of aflatoxin B_1 for varying periods. Repeated exposure over long periods of time increases toxin efficacy as a carcinogen (Table 2). Rainbow trout are extremely susceptible to tumor development aflatoxin ingestion. Toxin levels of 0.5 ppb in the feeding ration administered for 12 months to test trout have induced a 32% incidence of hepatomas (20).

TABLE 2—Tumor incidence in rats fed an Aflatoxin B_1-Contaminated ration

Aflatoxin B_1 level in ration (ppm)	Length of Feeding (weeks)	Aflatoxin B_1 ingested (mg)	tumor frequency (%)
1.0	35	2.9	80
0.3	35	1.0	14
0.3	52	1.4	38
0.015	52	0.07	0
0.015	68	0.095	100

Source: Reference 66.

NATURAL OCCURRENCE OF AFLATOXIN IN AGRICULTURAL COMMODITIES

Commodities contaminated with aflatoxin acquired from markets in the U.S. include: peanuts, cottonseed, corn, tree nuts and copra (14, 44, 59, 60, 61). In addition, concern has been growing about occurrence of the toxin in grain sorghum, rice and figs (14, 56). Peanuts have been the most thoroughly studied in terms of aflatoxin contamination. This accelerated research can be attributed to the initial identification of the problem in peanuts products and because peanuts are a major food. Certification of aflatoxin-free peanuts has been developed by FDA, USDA and the National Peanut Council. The procedure detects toxin-contaminated lots and diverts them to nonfood use. The incidence of aflatoxin in cottonseed and cottonseed products has also been extensively examined. Interestingly, the incidence of aflatoxin differs in samples from cotton-growing regions of southern California and from low altitude regions of Arizona (14). Although no reason for variations in toxin occurrence between regions has been established, apparently insect damage to the developing crop may be involved, and agronomic practices, including irrigation, are suspect since they could provide the humidity conditions conducive to *A. flavus* growth.

Several groups have concentrated intensive research on establishing a correlation between aflatoxin in human food and occurrence of cancer. Purchase and Goncalves (40) examined foods ingested by Bantus in an area of Mozambique where liver cancer is common. Corn, peanuts, rice, manioc and nuts are the staple foods in the area. Samples of cooked and uncooked commodities were collected from villages. Aflatoxin was detected in peanuts, corn, rice and manioc at levels exceeding 1000 ppb (Table 3). Alpert and his coworkers (3) investigated native foods in Uganda. Of 476 samples gathered during 1966–67, about 20% contained detectable levels of aflatoxin and 3.0% contained 500 ppb levels or higher. Frequency of contamination and also levels of toxin were high in peanuts, beans, corn, and sorghum (Table 3). Alpert's studies indicated a distinct variation between districts of the country in the occurrence of aflatoxin. Examination of hospital records suggested an association between regional incidence of hepatomas and aflatoxin contamination of foods.

For several years a research group from MIT has extensively studied the relationship between dietary intake of aflatoxin and human hepatoma in Thailand. For 2 years, more than 2000 samples of 170 varieties of foods and foodstuffs were collected throughout the country from markets, mills, warehouses, distributors, farms and homes (45, 46). About 10% of all the samples were contaminated with aflatoxin, with peanuts and corn exhibiting the highest incidence and toxin levels (Table 3). These investigations provided presumptive information linking hepatoma and aflatoxin contamination of food.

Campbell and his colleagues (8, 9) studied the occurrence of aflatoxin in raw and processed foods from areas of the Phillippines where liver malignancies are common. One of the unusual aspects of their studies was the discovery of enhanced levels of aflatoxin in commercial peanut butters (Table 3). They also observed extensive contamination of corn and corn products. In an important investigation of human subjects ingesting food contaminated with aflatoxin, the hydroxylated toxin, M_1, was found in urine and in mother's milk (8, 9). A correlation was observed between ingestion of aflatoxin B_1 and excretion of M_1. The hydroxylated derivative was detected in urine when total ingestion of B_1 exceeded 15 μg/day.

Identification of aflatoxin-contaminated corn in preliminary U.S. surveys and FDA action (15, 39) aroused considerable concern in the agriculture research community. Initially, the problem in corn was considered exclusively associated with improper storage of the com-

TABLE 3—Aflatoxin B_1 contamination of selected foods

Country	Commodity	No. Samples (contaminated/examined	Average aflatoxin B_1 in positive samples ($\mu g/kg$)
South Africa	Peanuts	5/67	2 Samples >1000
	Corn	2/52	>1000
	Rice	1/23	>1000
	Manioc	1/8	>1000
	Total	9/150 (6%)	
Uganda	Beans	15/64	500
	Peanuts	29/150	363
	Corn	19/48	133
	Sorghum	16/69	152
	Cassava	2/34	879
	Millet	6/55	26
	Peas	2/19	30
	Sin-sin	2/11	33
	Food mixture	2/15	73
	Rice	0/11	0
	Total	93/476 (20%)	
Thailand	Peanuts	116/216	872
	Corn	22/62	265
	Chili pepper	12/106	80
	Millet	5/44	38
	Dried fish/shrimp	7/139	104
	Mung beans	7/140	ca. 10
	Beans, general	10/322	106
	Cassava	2/65	60
	Rice	7/364	ca. 10
	Total	188/1458 (13%)	
Philippines	Peanuts, whole	80/100	98
	Peanut butter	145/149	213
	Peanut candies	47/60	38
	Corn, whole	95/98	110
	Corn products	22/32	32
	Rice products	1/72	<1
	Root and tuber products	48/62	44
	Beans	26/29	35
	Commercial livestock feeds	39/42	28
	Total	503/644 (78%)	

Source: References 3, 8, 9, 40, 45, 46.

modity, and preventive procedures were based on avoiding seed damage in harvest and transport and on appropriate drying (57, 58). ARS workers at the Northern Regional Research Laboratory, Peoria, Ill., carried out surveys of the 1964 and 1965 crop of corn from the Midwest; out of 1311 samples, 30 or 2.3% contained the toxin—all in grade 5 or Sample Grade (49). A similar examination of corn from the 1967 crop showed that 6 of 283 samples (2.1%) contained toxin—all in grade 3 or Sample Grade (51). In a subsequent study of 293 samples of export corn collected at 10 ports, 8 samples contained toxin at levels ranging from 3 to 27 ppb B_1 and, except for a single sample from U.S. No. 2 corn, all toxin positives were found in lower grades (52). In a limited examination of 1969 and 1970 corn from Alabama, North Carolina, South Carolina, Tennessee and Virginia, 21 of 60 samples contained aflatoxin and 12 samples exceeded 20 ppb of B_1 (50).

FDA seizure of a corn product in 1971 involved corn that had been grown in southeastern Missouri (1). Extent of the contamination was examined through a cooperative venture between ARS, ASCS and CCC. Approximately ½ million bushels of corn under loan in the region was accepted for delivery during 1972 and analyzed for aflatoxin after CCC had assumed ownership of the commodity. Aflatoxin was detected in 30% of the 1283 truckloads of corn; 93 trucks, 10–19 ppb; 45 trucks, 20–29 ppb; 91 trucks, 30–100 ppb; and 29 trucks above 100 ppb (53).

Indentification of alfatoxin in Missouri corn raised several interesting questions concerning the origin of the *A. flavus* inoculum. Although the consensus view held that the aflatoxin contamination occurred in storage, several observations suggested that the fungus might have occurred in the field (7, 14). Studies carried out in the 1920's showed that *A. flavus* was capable of invading developing corn ears during the milk stage (55). *Aspergillus* infections were generally associated with insect damage, particularly activities of the corn earworm *Heliothis zea* (Boddie). Field studies had also shown that *A. flavus* invaded peanuts and cotton bolls in the field. These observations prompted reconsideration of the extent that *A. flavus* might infect pre-harvest corn.

Broad surveys required development of a simple, rapid, presumptive test that could be used to detect samples suspected of aflatoxin contamination. Marsh and coworkers (33, 34) had extensively studied the relationship between the appearance of a bright greenish-yellow (BGY) fluorescence in cotton fibers and the occurrence of *A. flavus* in the

fluorescence in cotton fibers and the occurrence of *A. flavus* in the fluorescing spots. Investigation of the origin of the BGY-fluorescing material demonstrated that it was not aflatoxin but rather a derivative of kojic acid, another fungal metabolite (5, 34). After preliminary observations of a UV-induced fluorescence associated with aflatoxin-contaminated corn, a screening procedure was proposed based on BGY fluorescent emission (48). Although the test is not conclusive, it has been used widely as a preliminary screening tool.

In the fall of 1972, a study of field corn in southeast Missouri and east central Illinois was initiated; the objectives of the investigation were: (a) determine incidence of BGY fluorescence and insect damage on freshly harvested corn; (b) study occurrence of BGY fluorescence and aflatoxin after drying, shelling and cracking of corn from individual test ears; and (c) examine factors that might contribute to fungal infection and toxin formation. In a general survey, 60 ears were collected from each of 60 fields, but a few fields were intensively examined: 600 ears from two Missouri fields and 750 ears from five Illinois fields. Fluorescence on the freshly harvested ears was generally observed on ear tips. The bulk of this fluorescence occurred on immature seed; we estimated that only 1% of the samples contained BGY fluorescing kernels that could be considered mature (30).

After shelling, drying and cracking, 237 samples of the 3600 ears in the general survey and 12 of the 1350 ears in the intensive study exhibited BGY fluorescence (Table 4). Aflatoxin tests showed that 120/3600 in the general survey and 6/1350 in the intensive study contained aflatoxin at levels exceeding 20 ppb B_1. Of all the test ears collected, 2.5% contained toxin above the 20 ppb level. Distinct regional differences were observed in toxin occurrence. European corn borer and corn earworm were the predominant types of insect damage observed on sample ears. Corn borer damage was detected on 21% of the Missouri general survey, 12% of the Missouri intensive and 17% of the Illinois corn. Corn earworm was observed on 21% of the Missouri general survey, 25% of the Missouri intensive and only 5% of the Illinois samples. According to a statistical analysis, the incidence of aflatoxin-positive corn was significantly higher on ears with earworm damage than on those without.

From the sample ears, 195 insects were collected, placed in sterile vials and subsequently plated on a nutrient medium (16). Of these insects, 29 yielded *A. flavus* colonies and from these, 25 isolates were aflatoxin-producing strains. Other fungi commonly observed on the

TABLE 4—Incidence of bright-greenish yellow (BGY) fluorescence and Aflatoxin in the general and intensive corn surveys of Missouri and Illinois corn

Survey area	Number of sample ears	Incidence[a]	
		BGY positive	Aflatoxin B_1 >20 ppb
General			
1	900	84	39
2	900	133	73
3	900	14	5
4	900	6	3
Total	3600	237	120
Intensive			
5	600	12	6
6	750	0	0
Total	1350	12	6

Source: Reference 30.

[a]Incidence based on fluorescent and aflatoxin positive samples from individual ears.

insects included strains of *Fusarium, Rhizopus, Penicillium* and *Mucor.*

Since aflatoxin occurrence in freshly harvested corn was unexpected, all procedures were carefully reviewed for possible post-harvest production of the toxin. Delineation of field and post-harvest contamination was compromised by the method used in drying samples. Husked ears had been examined and placed in forced-draft 60 C dryers within 8–12 hours after removal from the stalks. However, the time between entry of test ears into the dryer and achievement of tempertures prohibiting further *A. flavus* development (46–50 C) was not recorded. Therefore, the test could not exclude the remote possibility that aflatoxin contamination occurred or was extended in the 24–48 hours immediately after harvest.

To acquire more definitive information on the occurrence of aflatoxin in corn before harvest, and to minimize the time between sample collection and drying, high-capacity, forced-draft dryers were operated at 90 C. Under these conditions, 20–25% moisture corn could be dried to 12–14% within 3–6 hours with no appreciable destruction of aflatoxin.

A sampling region in South Carolina was selected for an experiment in 1973 since FDA has seized aflatoxin-contaminated corn earlier that year from the area (61). There were collected 297 ten-pound samples

in the field and at delivery points during harvest. Samples were placed in dryers within ½ to 4 hours after collection (31). of the 184 samples taken directly from the field, 92 contained detectable levels of toxin; 62/184 (34%) contained toxin above 20 ppb with six specimens exhibiting B_1 above 160 ppb (Table 5). Of the 113 samples taken at elevators, 60

TABLE 5—Levels of Aflatoxin in corn samples from South Carolina

Aflatoxin B_1 (ppb)	Number of Samples				
	Field		Elevator		
	Picker-Sheller	Truck	A.M.	P.M.	Total
ND[a]	46	46	34	19	145
⩽9	6	6	3	6	21
10–19	8	10	13	6	37
20–39	8	16	11	3	38
40–79	8	10	5	4	27
80–159	5	6	3	4	18
160–319	3	3	1		7
320–639		3			3
>640			1		1
Total	84	100	71	42	297

Source: Reference 31.
[a]Not detected.

contained detectable levels of toxin; 32/113 (28%) contained toxin above 20 ppb with one sample exhibiting B_1 above 640 ppb. Of the aggregate samples, 94/297 (32%) were contaminated with aflatoxin B_1 above 20 ppb.

Mycological studies were also carried out on the corn samples from South Carolina and on the insects collected from them. Of the 152 aflatoxin-positive samples, 120 showed one or more kernels internally infected with *A. flavus* utilizing 50 kernels/sample as the test unit (21). In a similar examination, 59 of the 145 aflatoxin-negative samples were internally infected. Of the aggregate 297 samples, 276 had one or more kernels exhibiting *A. flavus* on the seed surface. In 75 samples all 50 test kernels were surface-contaminated with the fungus. Out of the 375 insects collected, 274 contained *A. flavus*. Because 78 of 85 rice weevils exhibited *A. flavus*, it was concluded that this insect might be involved in the infection of developing corn ears with the toxin-producing fungus.

FACTORS INFLUENCING AFLATOXIN PRODUCTION IN AGRICULTURAL COMMODITIES

Consideration of the causes of aflatoxin contamination of a commodity invariably leads to examination of factors that modify fungal development particularly moisture, temperature, aeration and the substrate (14). Probably moisture is the most important single environmental component controlling fungal growth. Largely based on moisture requirements, fungi that occur in seed have been roughly grouped into genera that occur in the field and those that occur primarily in storage (11). Routinely, field fungi invade seed at moisture levels exceeding 20%; the major genera in this category include *Alternaria, Helminthosporium, Fusarium* and *Cladosporium*. Storage fungi predominate on grain in the 13 to 18% moisture range. These fungi are principally species of *Aspergillus* and *Pennicillium*. Representatives of the *Aspergillus glaucus* group do not produce aflatoxin; they are generally found on grain at 13 to 15% moisture, but above 15% other fungi including *A. flavus, A. ochraceus* and *A. versicolor* are observed (12). In response to observations relating moisture content to fungal growth, the U.S. Department of Agriculture has published a bulletin describing drying techniques for control of mycotoxin contamination recommending that corn be dried to 13% moisture within 24 hours after harvest (58).

Generally, fungi grow readily between 20 and 30 C; however, the absolute temperature limits can range from below freezing to 60 C (14, 28). Temperature affects growth rate of microbes as well as type of metabolic products formed. Laboratory studies have described maximum aflatoxin yields at 24 to 25 C with some synthesis between 11 and 37 C (14), but other reports have related optimal production of the toxin as 35 C (14). Apparently, temperature requirement for maximum aflatoxin synthesis depends on the unique properties of the test system.

Although fungi are aerobic organisms, significant differences have been observed for oxygen requirements among different species. The gaseous environment influences physiology of the fungus and, subsequently, synthesis of metabolites (28). Reducing oxygen from 5 to 1% dramatically inhibits growth of *A. flavus* and aflatoxin formation (14).

The substrate is also a determining factor in development of *A. flavus* in a natural ecological milieu. In laboratory studies wide arrays of grains, oilseeds, forages, raw and processed foods support *A. flavus*

growth and toxin production (14). Nutritional investigations have shown that simple carbon and nitrogen sources, plus additional mineral salts, provide the necessary ingredients for growth of the fungus and synthesis of aflatoxin (14). Condition of the substrate can also be critical in establishing a natural fungal infection. Physical modification of the substrate, such as stress cracks and breakage in grain, predisposes the seed to fungal infestation (57). Reducing grain damage during harvesting and drying is essential to subsequent control of fungal infection in a stored commodity.

There must be a primary inoculum present before fungal infection can occur. Fungal spores, including *A. flavus,* are generally abundant in soil, air and water; however, environmental factors determine the successful establishment of particular fungal species. Low-grade grain heavily contaminated with fungi represents a source of fungal propagules; this primary inoculum potential can be particularly hazardous when lots of corn are blended.

FACTORS INFLUENCING AFLATOXIN PRODUCTION IN CORN BEFORE HARVEST

Field investigations had shown that *A. flavus* can infect corn before harvest (41, 42, 55). However, studies in central and northern regions of the U.S. identified only a limited incidence of the fungus (11, 41). Other studies had provided presumptive evidence for regional differences in *A. flavus* infection of field corn with indications that corn grown in warm, humid areas was particularly susceptible (7). Laboratory studies have presented evidence for broad varietal differences in resistance to the toxin-producing fungus (37, 38).

In response to *A. flavus* infection in preharvest corn in 1972, field experiment was designed for the following year to study: (a) aflatoxin production variation in *A. flavus*-inoculated ears of field corn grown at geographically diverse locations, (b) aflatoxin incidence in mechanically damaged ears of field corn, (c) differences in susceptibility between two endosperm types of two corn hybrids to *A. flavus* production of aflatoxin under field conditions, and (d) aflatoxin production at varying times after inoculation of field corn with *A. flavus.*

Normal and *opaque-2* counterparts of a white and yellow hybrid were grown at Tifton, Georgia; College Station, Texas; Columbia, Missouri; and Peoria, Illinois. Ears were inoculated 20 days after silking by insertion of a hypodermic syringe needle through the husk into the

region of the developing kernels and dispensing a measured volume of an *A. flavus* inoculum. Test ears were harvested 15, 30, 45 and 70 days after treatment. The number of aflatoxin-positive ears increased generally from north to south: Illinois, 22%; Missouri, 67%; Texas, 87%; and Georgia, 91% (32). The mean level of aflatoxin B_1 in toxin-contaminated ears showed a similar geographical pattern: Illinois, 2.4 ppb; Missouri, 22.5 ppb; Texas, 114.5 ppb; and Georgia, 133.9 ppb (Table 6). Hybrid I had significantly higher levels of aflatoxin than Hybrid II; no difference was observed in normal versus *opaque* types. Most of the toxin production occurred during the first 30 days after inoculation. Twelve percent of the physically damaged ears and 4% of

TABLE 6—Levels of Aflatoxin B_1 in corn samples of ears inoculated with *Aspergillus flavus* and grown at diverse locations

	Experimental Observations	Aflatoxin B_1 (ppb—Mean[a])
Hybrid I		
	Hybrid	
	I	59.8
	II	15.3
	LSR[b]	1.5
	Endosperm type	
	Normal	24.2
	Opaque-2	37.8
	LSR[b]	1.5
	Time after inoculation, days	
	15	11.7
	30	36.8
	45	45.2
	70	43.2
	LSR[b]	1.9
	Location	
	Illinois	2.4
	Missouri	22.5
	Texas	114.5
	Georgia	133.9

Source: Reference 32.

[a]Aflatoxin B_1 levels are presented as geometric means of aggregate corn samples from ears inoculated with *A. flavus*. The geometric mean is the antilogarithm of the logarithmic mean of aflatoxin B_1 concentrations.

[b]LSR is the least significant ratio (5% level) of two means.

the untreated ears contained aflatoxin. More than 90% of the damaged and control ears contaminated with toxin came from Georgia and Texas plots (32). The study provided unequivocal evidence that *A. flavus* infects developing corn if the fungus is introduced into the region of developing kernels. Since aflatoxin incidence and levels were highest in corn from southern locations, the results suggested that regional differences might be critical in determining the extent of fungal infection and toxin synthesis in preharvest corn.

In similar studies, Anderson et al. (4) also observed a higher incidence of aflatoxin in corn grown in warmer, more humid regions. Examination of factors contributing to the problem showed that growing corn under stress conditions, such as dense populations of plants or reduced fertilization, appeared to increase the incidence of aflatoxin contamination. Study of strain differences in susceptibility to *A. flavus* infection also showed no resistance inherent in *opaque-2* types. Investigation of the seed used in the tests demonstrated that one of the strains contained low levels of the toxin. However, presence of the toxin appeared to have had no adverse effect on subsequent development of the corn (4).

Further examination of strain differences in *A. flavus* susceptibility was carried out in a 1974 study of corn grown in South Carolina and Florida. The investigation was designed to evaluate differences between corn strains adapted and those not adapted to growth in the southern U.S. The relationship between insect activity and toxin contamination was also studied. The test hybrids included five experimental South Carolina (SC) single crosses varieties adapted to growth conditions in the southern U.S. and a commercial, single cross hybrid (A) adapted for the Corn Belt but widely grown in the South. At both locations, BGY occurrence and toxin levels were lower in SC hybrid corn than in hybrid A samples (Table 7). Insecticide treatment reduced but did not eliminate the toxin in all the test varieties. In this study developing ears were inoculated with *A. flavus* spores by introducing the inoculum into the silk bundle. Therefore, differences in susceptibility to the fungus could reflect simply a difference in husk protection from insect attack and associated introduction of the spores into the kernel region of the ear.

Detailed investigations of the relationship between insect activity and aflatoxin contamination of corn have been carried out by Widstrom et al. (62, 63). Hand infestation of developing ears with corn earworm; fall armyworm, *Spodoptera frugiperda* (J. E. Smith); and European corn borer, *Ostrinia nubilalis* (Hubner) produced conditions conducive

TABLE 7—Summary of occurrence of BGY fluorescence and Aflatoxin levels in ears of test corn grown in South Carolina and Florida[a]

	South Carolina		Florida	
Hybrid	BGY (%)	Aflatoxin B_1 (ppb—Geometric mean)	BGY (%)	Aflatoxin B_1 (ppb—Geometric mean)
SC76 X SC413	2 cd	1 d	8 c	3 c
SC44 X SC413	11 b	29 b	14 b	66 b
SC441 X SC413	5 b	8 c	5 c	3 c
SC31 X SC413	6 bc	6 c	11 bc	63 b
SC403 X SC401	4 bc	7 c	8 c	30 b
A	45 a	230 a	45 a	1564 a
Treatment				
Control	11 b	18 b	8 b	26 b
Insecticide	2 a	3 a	10 b	8 a
A. flavus inoculated	18 c	56 c	27 a	165 c
Damage	11 b	8 b	16 c	62 bc

[a]BGY occurrence and aflatoxin levels were obtained from a total of 70 ears from South Carolina and 56 ears from Florida. Aflatoxin B_1 levels are presented as the geometric means; this value is the antilogarithmic mean of aflatoxin B_1 concentrations. Values within a column in the table with no letter in common differ significantly at the 5% level.

to aflatoxin production. Ears infested with European corn borer had the highest concentration of aflatoxin B_1 at harvest. Intensive application of insecticide to developing ears reduced but not eliminated insect damage, neither did such treatment effectively preclude *A. flavus* infection and subsequent aflatoxin production.

COPING WITH AFLATOXIN CONTAMINATION OF CORN

Two broad concepts answer two aflatoxin problems: (a) How do we avoid it? Answer, prevention; and (b) what do we do with a toxin-contaminated commodity? Answer, proper management.

Prevention of aflatoxin development can be arbitrarily divided into control of *A. flavus* during preharvest and in conditions after harvest. Previous suggestions to prevent aflatoxin development in corn dealt primarily with enhancing the quality of storage. Recommendations included: (a) appropriate harvest and transport procedures to minimize kernel breakage, (b) prompt drying of the commodity to safe levels

(13%), (c) adequate storage aeration to avoid hot-spots, and (d) elimination of blending fungal-contaminated corn with high-quality lots of grain (29, 57, 58).

The latest studies identifying extensive *A. flavus* infection of corn in the field have prompted an extension of prevention procedures. Available research information does not provide an unequivocal basis for establishment of prevention techniques in the field. Current awareness of the broad occurrence of *A. flavus* in field corn in certain regions should stimulate increased concern for appropriate harvesting and storage of the commodity. A large indigenous inoculum of the toxin-producing fungus clearly increases the hazard of contamination of freshly harvested corn (54). Obviously, the best method of coping with the potential health hazard associated with aflatoxin contamination of foods and feeds is to prevent *A. flavus* development. In the absence of procedures that eliminate the problem, consideration must be given to effective management of toxin-contaminated materials (19). Techniques have been developed for detoxification of aflatoxin in various commodities; these include: (a) removal—physically, chemically or biologically—and (b) inactivation—physically or chemically.

The rationale for physical separation of aflatoxin-contaminated seeds from larger quantities of material is based on the recognition that the toxin is often found in a small percentage of individual seeds of the adulterated commodity. The peanut industry has for some time employed selection techniques designed to cull undesirable seed (24). Manual, mechanical and electronic selection procedures have been employed to exclude peanuts that are discolored, damaged or inadequately developed. Although physical separation expedites reduction of toxin levels to some extent, apparently a number of sound kernels contain the toxin (14). Physical separation in corn as a detoxification technique has also been investigated (6); the procedures did not eliminate all the toxin-contaminated kernels in a contaminated lot. Wet milling of corn containing aflatoxin showed that 80–90% of the toxin was isolated in the gluten feed fraction (steepwater, fiber and spent germ) (67).

Removal of aflatoxin from contaminated commodities by chemical methods has generally involved solvent extractions; some success has been achieved in these tests but they are cumbersome, expensive and generally not totally satisfactory (14). Certain microbes have also been identified as detoxifying agents, but the associated procedures are not economically feasible (14).

Heat has been used as a technique for physical inactivation of aflatoxin (14). The toxin is relatively heat-stable at 120–150 C, but higher temperatures have been employed in roasting procedures to reduce toxin levels (47).

Chemical inactivation is the most widely studied detoxification procedure. Alkali, acid, propylene oxide, sulfur dioxide, chlorine, ozone, sodium hypochlorite, hydrogen peroxide and other chemicals have been tested (14). The substance that appears to offer the greatest practical potential for large-scale detoxification is anhydrous ammonia. A method has been developed for detoxification of contaminated peanut meal through treatment with ammonia gas under pressure (40–50 psi) at 90–125 C (14). A research group at the Northern Laboratory is examining the efficacy of ammonia in a closed system without enhanced pressure levels as a method for detoxifying corn (13).

With the exception of dairy cows, a potential biological detoxification procedure involves feeding a toxin-contaminated commodity to mature, domestic animals. Early studies demonstrated that the following levels of aflatoxin B_1 could be fed without significant reduction in feed efficiency or detectable residues of the toxin in tissues: swine, 223 ppb; cattle, 300 ppb; and chickens, 500 ppb (18, 22, 25, 26). However, later studies showed that residues are present in small quantities in tissues from animals consuming relatively high levels of toxin (Table 8) (23, 27, 35, 36). Comparison of the 4 ppb concentration of aflatoxin M_1 in milk from cows fed 700 ppb B_1 with residues in other tissues demonstrates the need for restriction of aflatoxin-contaminated rations in dairy cows (Table 8). Within the defined constraints, the general

TABLE 8—Aflatoxin residues in animal tissues.

Animal [a]	Feeding (days)	Aflatoxin B_1 in feed (μg/kg)	Tissue Residues: Aflatoxin B_1 (μg/kg) Muscle	Liver	Eggs	Aflatoxin M_1 (μg/kg) Milk
Swine	166	400	1	12		
Cattle	14	700	ND	1		
Cows	7	700				4
Broilers	56	1000	0.01	0.25		
Hens	15	400			1.4	

[a] Source references: Swine (27), cattle (35), cows (35), broilers (36) and hens (23).

observations support the view that animals can consume rations containing up to 200 ppb B_1 with no adverse effects either in feed efficiency or in accumulation of toxin residues to levels representing a health hazard. However, FDA has established a guideline level of 20 ppb aflatoxin B_1 in corn (15, 61).

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